American Fern Journal 89(4):221–231 (1999)

Interpopulational Comparison of Dose-Mediated Antheridiogen Response in *Onoclea sensibilis*

RICHARD D. STEVENS and CHARLES R. WERTH
Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409

ABSTRACT.—Intraspecific variation in antheridiogen response has been reported in several fern species. Here, we characterized and compared dose-mediated antheridiogen response among six populations spanning the range of *Onoclea sensibilis,* a species widely used for antheridiogen assays. A crude aqueous filtrate (CAF) of antheridiogen A_{PT} was obtained from cultures of *Pteridium aquilinum* and introduced into mineral agar at six concentrations ranging from 0 (control) to 10^{-1}. Response was initially characterized for one population using three criteria: percentage of male gametophytes, number of antheridia per gametophyte, and mean surface area of gametophytes. Percent male was consistently the best descriptor of the response profile and was used to compare responses among populations. In all populations, gametophytes exhibited little or no response at CAF concentrations 10^{-5} and lower; a saturated response, almost all gametophytes being male at 10^{-3} and higher; and an intermediate response, usually between 50% and 80% males, at 10^{-4}. With few exceptions, response levels and profiles were very consistent among populations. Data and methodology provided herein indicate that use of the percent male criterion can provide a reliable quantitative assay using *Onoclea* that can facilitate future comparative research in antheridiogens.

Gametophytes of numerous and diverse taxa within the Polypodiaceae sensu lato share response to antheridiogen A (sensu Voeller 1964; in this paper antheridiogen A refers to the general class of antheridiogens produced by and biologically active in taxa belonging to Polypodiaceae sensu lato, whereas antheridogen A_{PT}, sensu Näf [1979], refers specifically to antheridiogen A produced by *Pteridium aquilinum* (L.) Kuhn). However, antheridiogen response is neither pervasive nor equivalent among polypodiaceous ferns. Species representing different genera may differ by orders of magnitude for the minimum A_{PT} dose required for response (Näf et al., 1975; Näf, 1979), and congeneric species may also differ in antheridiogen response, as shown in *Bommeria* (Haufler and Gastony, 1978), *Cystopteris* (Haufler and Ranker, 1985), and polystichoid ferns (Yatskievych, 1993). Variation in response may also occur within species, either among populations or even among individuals within populations. In *Hemionitis palmata* L., lower response was detected in populations with lower genetic load (Ranker, 1987), suggesting that reduced response to antheridiogen may facilitate a higher selfing rate, thereby eliminating recessive lethals within populations (Schneller et al., 1990). A genetic basis for variation in antheridiogen sensitivity was determined in *Ceratopteris.* Genotypes with lowered antheridiogen response were obtained experimentally in *C. richardii* Brongn. through application of mutagens, and a simple two-gene basis of inheritance for antheridiogen sensitivity was determined (Scott and Hickock, 1987; Warne et al., 1988).

These observations combine to indicate that antheridiogen response is sub-

ject to evolutionary change. Because antheridiogen may be of importance in determining or facilitating the mode of sexual reproduction by gametophytes, evolutionary changes in antheridiogen response may represent variation in an important life history attribute.

Of the species compared by Näf (1979), the greatest sensitivity to antheridiogen A_{PT} was exhibited by *Onoclea sensibilis* L., which was therefore used as a reference for interspecific comparisons and has become the standard fern species for assaying antheridiogen A. This species is broadly distributed, occurring over most of eastern North America and disjunct in eastern Asia (Gastony and Ungerer, 1997). Although geographic sources of *O. sensibilis* have varied among studies, the possibility of geographically based variation in antheridiogen response has not specifically been addressed. Most workers utilizing *O. sensibilis* (e.g., Haufler and Ranker, 1985; Miller, 1968) have reported highly sensitive antheridiogen response and lack of spontaneous (i.e., without exposure to antheridiogen) antheridium formation, comparable to that indicated by Näf (1979). However, unusual responses have been reported from occasional frond collections (Näf, 1979), for example spontaneous antheridium formation (Klekowski and Lloyd, 1968; Miller and Miller, 1970; Rubin and Paolillo, 1983). Furthermore, the way in which response levels in relationship to antheridiogen doses have been quantified has varied among studies and sometimes has not been fully explicit. The objectives of this study were to determine consistent criteria for quantifying antheridiogen response of *O. sensibilis* and to determine whether there are differences in response among populations from different regions.

Materials and Methods

The antheridiogen exudate used in this study was obtained from gametophytes of *Pteridium aquilinum*, a standard source of antheridiogen A due to the high activity of its exudate. Spores from *P. aquilinum* accession *Sheffield 56* from England were sown under axenic conditions as follow. Spores were separated from sporangia by rinsing through a Nitex screen with 100 μm openings, using a wetting solution (2 drops of Tween-80 per 100 ml distilled water). Spores were collected in a cylindrical "basket" made from an inverted plastic 30 mm centrifuge tube. The conical bottom of the tube was cut off, and the cap was perforated with numerous holes using a hot dissecting needle. A fine-meshed Nitex screen (5 μm openings) was placed between the cap and the centrifuge tube opening to form a "basket" bottom that would retain spores but allow passage of liquids.

Spore samples, wet with the Tween solution, were rinsed three times with distilled water and set in the dark overnight to allow germination of fungal spores. The following day, the baskets were placed in a sterilizing solution of 5% commercial hypochlorite for 90 sec, then placed in three rinses of sterile distilled water. Spores were then sown by pipetting onto mineral agar in densities ranging from 5–30 spores per cm^2 in plastic petri dishes.

Mineral agar contained Parker's macronutrients and Thompson's micronu-

TABLE 1. Collection localities for spores of *Onoclea sensibilis* used in this study. Collectors are indicated in parentheses.

Population	Location
Gray, GA	Georgia: Jones County, U.S. 129 South of Gray (C. R. Werth)
Vienna, VA	Virginia: Fairfax County, along W&OD trail near intersection with Gallows Road (C. R. Werth).
Nashville, TN	Tennessee: Davidson County, plant under cultivation, collected near Nashville (D. P. Whittier).
Berwick, PA	Pennsylvania: Luzerne County, Beach Haven, Salem township TR 438 (J. D. Montgomery)
Richmond, VT	Vermont: Chittenden County, 3 miles northeast of Richmond (C. A. Paris and D. Barrington)
Eagle, WI	Wisconsin: Waukesha County, Section 29, County Highway 5, 1 mile west–southwest of Eagle (W. C. Taylor)

trients, as described in Klekowski (1969). Media were autoclaved and poured into petri dishes. Petri dishes inoculated with spores were placed under continuous light of 45 micromole quanta provided by 40 watt cool-white fluorescent bulbs. The water in which the spores were introduced evaporated (or was absorbed by the agar) after one to two days. Petri dishes were then placed in sealable sandwich bags to minimize further evaporation.

Gametophytes of *P. aquilinum* were grown for approximately one month. The agar was then frozen and thawed, and the aqueous phase was separated from the agar matrix by filtration, yielding a crude aqueous filtrate (CAF). The present study used A_{PT} CAF 89–1 produced during the summer of 1989, which was stored frozen in 100 ml plastic bottles until use in the present series of experiments conducted over the years 1990–1992. Single bottles of antheridiogen extract were used for several experiments and these bottles were kept refrigerated after thawing and used in successive experiments. Antheridiogen activity was apparently stable over the duration of the experiments under these conditions, as no appreciable decrease in response was observed (see results).

Sporophylls of *O. sensibilis* were obtained between January and February (i.e., before spores were released) of 1990 and 1991 from various locations (Table 1). Spores were harvested from loose chaff or by inducing sporangial dehiscence. It was found that dehiscence could be induced by thoroughly soaking sporophylls in water, placing them on smooth paper, and allowing them to dry overnight. This procedure resulted in release of large quantities of spores with minimal amounts of sporangia (note: sporophylls collected in the fall did not release spores after soaking, implicating a role for vernalization and wetting in timely spore release in nature.) Spore collections represented pooled spores released from 5–20 separate sporophylls for each population except the Nashville, TN, site, from which a single sporophyll was obtained. Spores were sterilized and sown using the same technique as described above for *P. aquilinum*.

Antheridiogen enriched media were prepared by adding various amounts (10^{-1}–10^{-6}) of CAF prior to autoclaving. Unenriched media provided a control

for antheridiogen dose experiments. Cultures were scored at 21 days after sowing. Although axenic conditions were sought, fungal contamination was experienced in a sizable number of replicates at all A_{PT} concentrations, apparently resulting from use of age-weakened hypochlorite solution. To evaluate the influence of fungal contamination, we performed an analysis of covariance to determine differences between contaminated and uncontaminated replicates, contamination state (+, −) being the covariate. There were no significant differences in response (percent male—see below) between contaminated and uncontaminated replicates of the same CAF concentration ($P = 0.248$). Therefore, contaminated dishes were included in the data set, except for a very few where fungi had overgrown the gametophytes.

For initial experiments, thirty gametophytes per treatment were harvested in an unbiased fashion, mounted on microscope slides in a solution of 1 part Hoyers mounting medium and 1 part acetocarmine, and examined one day later under low power using a compound microscope. For each gametophyte, length and width were measured using an ocular micrometer, and the number of each kind of gametangium (i.e., antheridia or archegonia) was recorded. Later experiments were evaluated only for percentage of male gametophytes per culture. It was found that the addition of acetocarmine directly to cultures would both fix the gametophytes and stain gametangia, and that gametophytes could be scored reliably for sex expression under a dissecting microscope without removing them from the petri dish. In these latter experiments, 50 gametophytes per treatment were scored for sexual expression. All experiments were repeated at least once.

Results

Characterization of Response to Antheridiogen.—Consistent with most previous observations (Näf, 1979), *O. sensibilis* gametophytes grown on media lacking CAF (control cultures) failed to produce antheridia, with rare exceptions (a single male individual was observed in a control culture of the Gray, GA, population). Although not all individuals matured sexually during the three week experimental period, nearly all gametophytes in control cultures allowed to grow greater lengths of time ultimately became female, i.e., produced archegonia in the region of their meristematic notch. Male gametophytes, possessing antheridia scattered away from the meristematic notch, consistently appeared in cultures with 10^{-4} CAF and higher. Also consistent with previous reports (Döpp, 1950; Näf, 1979), differences in morphology were observed between male and female *O. sensibilis* gametophytes. Females were invariably cordate and larger than males, which tended to develop numerous marginal lobes and often were ameristic at higher A_{PT} concentrations (10^{-1} and 10^{-2} CAF). Frequently, in cultures that initially possessed 100% males (10^{-3} and 10^{-2} CAF, see below) but were allowed to continue growing past the sampling date, a small number of gametophytes became large and cordate in the fourth week or later after sowing. Microscopic examination revealed that these were hermaphrodites that had apparently switched from being male and had

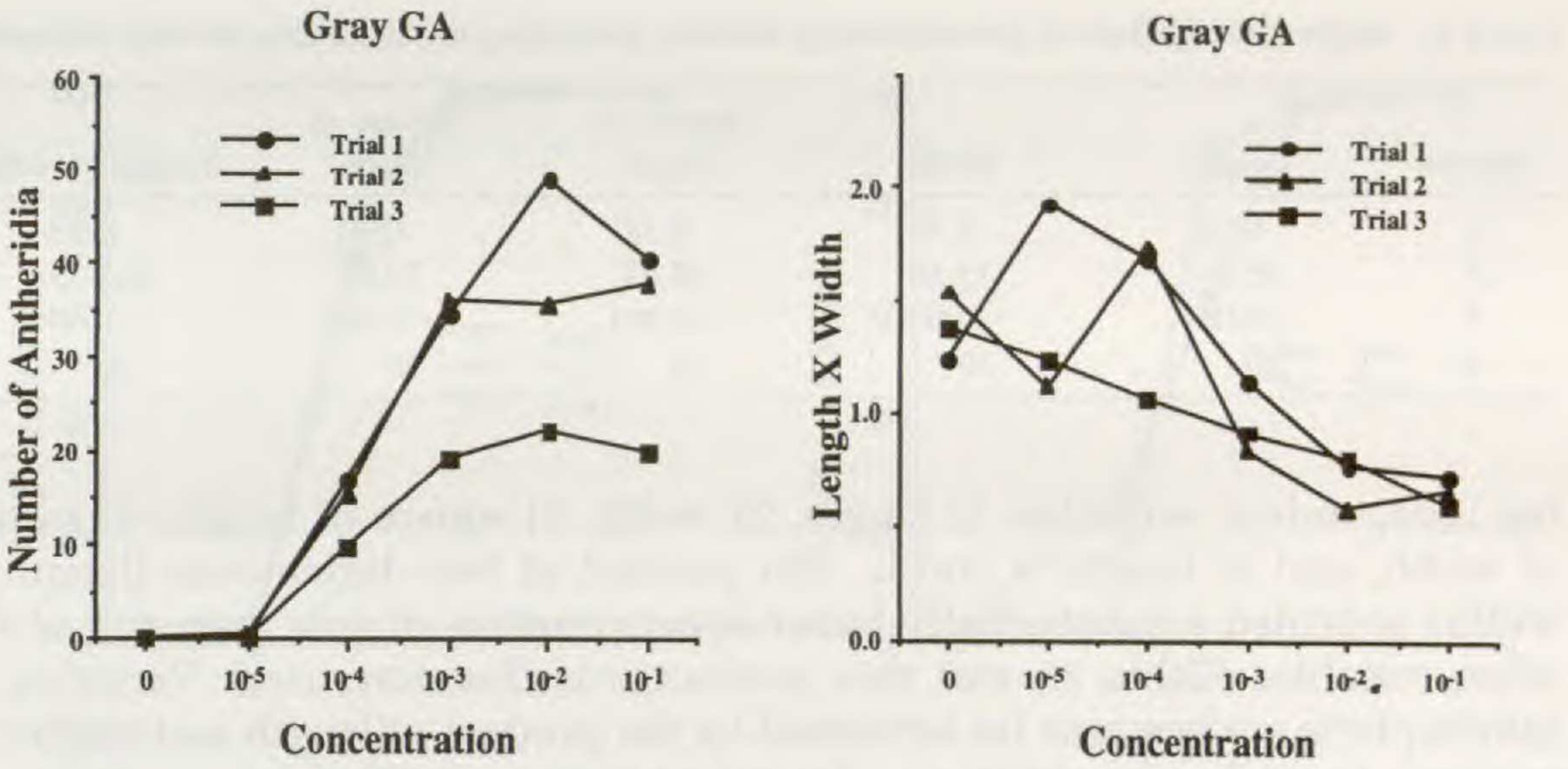


FIG. 1. Response of *Onoclea sensibilis* gametophytes from the Gray, GA, site to varied doses (i.e., CAF concentrations) of antheridiogen A_{PT}, as characterized by mean number of antheridia per gametophyte (left) and gametophyte surface area, estimated as length × width (right).

developed archegonia in the vicinity of their meristematic notches. The tendency for these hermaphrodites to appear seemed to be greater in 10^{-3} CAF cultures than in 10^{-2} CAF. Similar observations were reported by Näf (1979).

To determine the suitability of various criteria that could characterize and quantify antheridiogen response, initial experiments using a single accession (Gray, GA) evaluated three observable attributes of response by *O. sensibilis* gametophytes over A_{PT} concentrations of 10^{-1}–10^{-5} CAF. These attributes were: 1) mean number of antheridia per gametophyte, 2) mean surface area per gametophyte, and 3) percentage of male gametophytes.

Antheridiogen response characterized as mean number of antheridia per gametophyte is illustrated in Fig. 1A. Number of antheridia per gametophyte was very close to 0 in both the control and at 10^{-5} CAF. Maximum response was observed at concentrations of CAF 10^{-3} or higher. Two of the three replicates showed very similar response levels across CAF concentrations 10^{-1}–10^{-3}, suggesting that at this concentration mean number of antheridia exhibits a saturated response. However, the first replicate showed a substantially higher response at 10^{-2} than at either 10^{-3} or 10^{-1}. All three replicates showed an intermediate response at 10^{-4} CAF. In replicate 3, the mean number of antheridia per gametophyte was substantially lower than in the first two trials. This is believed to be due to differences in the spore sowing density.

The second criterion, gametophyte size, was evaluated because it is known to be affected by antheridiogen extracts (Näf, 1979) and to influence the number of antheridia per gametophyte, as demonstrated in *Cystopteris* (Haufler and Ranker, 1985). To determine the most effective means to estimate gametophyte surface area, gametophyte outlines were traced onto paper using a camera-lucida. These were cut out, their length and width measured, and their surface area determined using a leaf area meter. Area was regressed against the follow-

TABLE 2. Regression analyses of the relationship between gametophyte surface area and four estimators.

Statistic	Length	Width	Square of length	Square of width	Length × width
r^2	0.78	0.70	0.78	0.64	0.93
F	65.55	43.56	68.48	34.01	255.35
P	<0.001	<0.001	<0.001	<0.001	<0.001
N	20	20	20	20	20

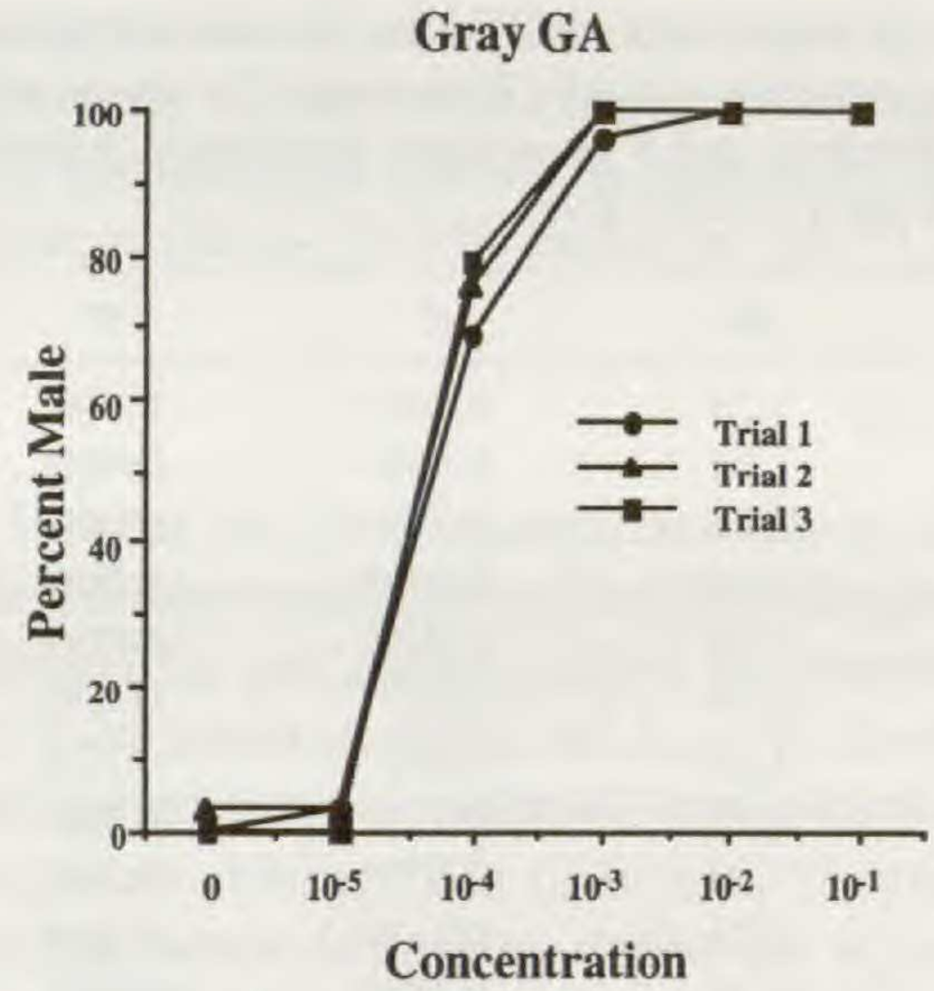
ing independent variables: 1) length, 2) width, 3) square of length, 4) square of width, and 5) length × width. The product of two dimensions (length × width) provided a substantially better approximation of area than any of the other variables (Table 2), and this product was therefore used. Variation in gametophyte surface area (as estimated by the product of length and width) in response to CAF concentration is illustrated in Fig. 1B. The area response was not as consistently predicted by CAF concentration as was either mean number of antheridia or percent male (see next paragraph), and furthermore was inconsistent across experiments. However, a tendency for area to decrease with increased CAF concentration was clearly indicated, consistent with previous reports (Näf, 1979).

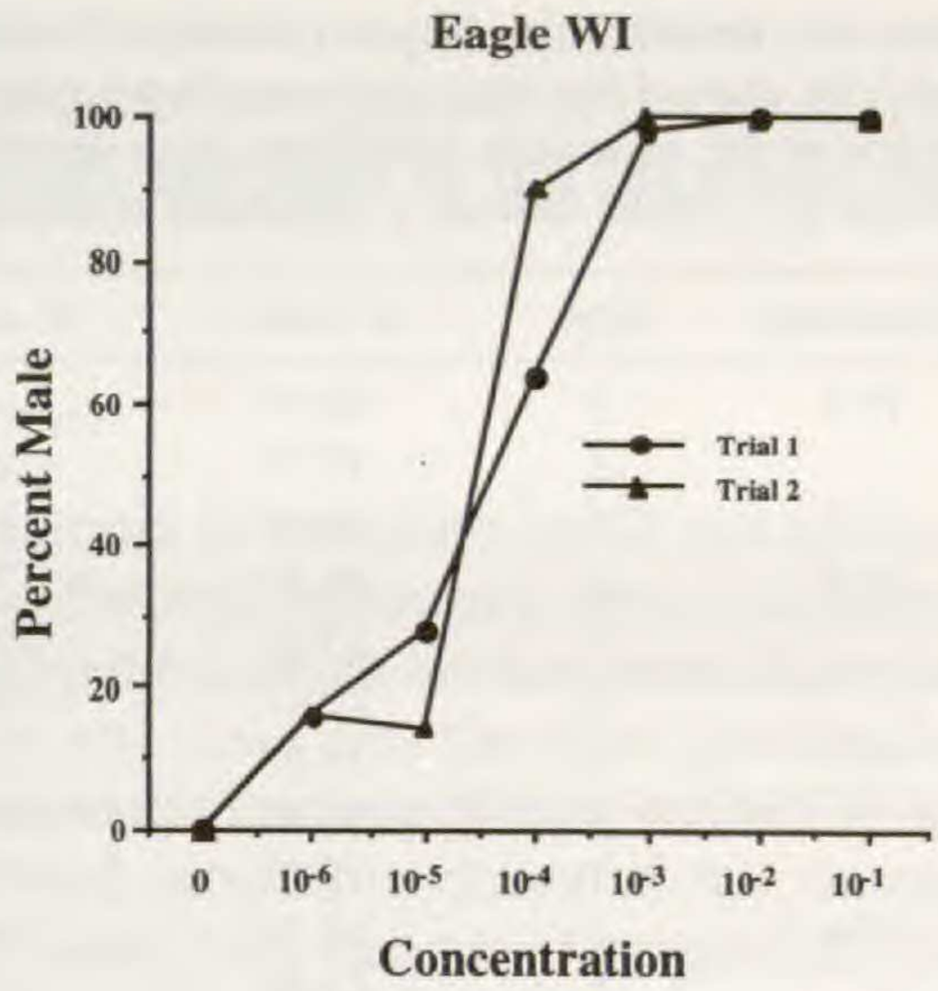
Antheridiogen response characterized as percent male is illustrated for the Gray, GA, population in Fig. 2A. Response at CAF concentration 10^{-5} was at or near zero and indistinguishable from the control. Response at CAF concentrations 10^{-1}, 10^{-2}, and 10^{-3} was at or near 100% males (96.5–100 %), indicating a saturated response for this criterion. At 10^{-4} CAF an intermediate response was observed (68.9–79.3% males), indicating that the threshold for substantial response lies between 10^{-5} and 10^{-4} CAF. This concentration seems highly comparable to Näf's (1956) determination of 3.2×10^{-5} as a response threshold concentration for A_{PT} exudate. Values for percent male at each concentration were highly consistent across the three replicates, much more so than for either number of antheridia or area.

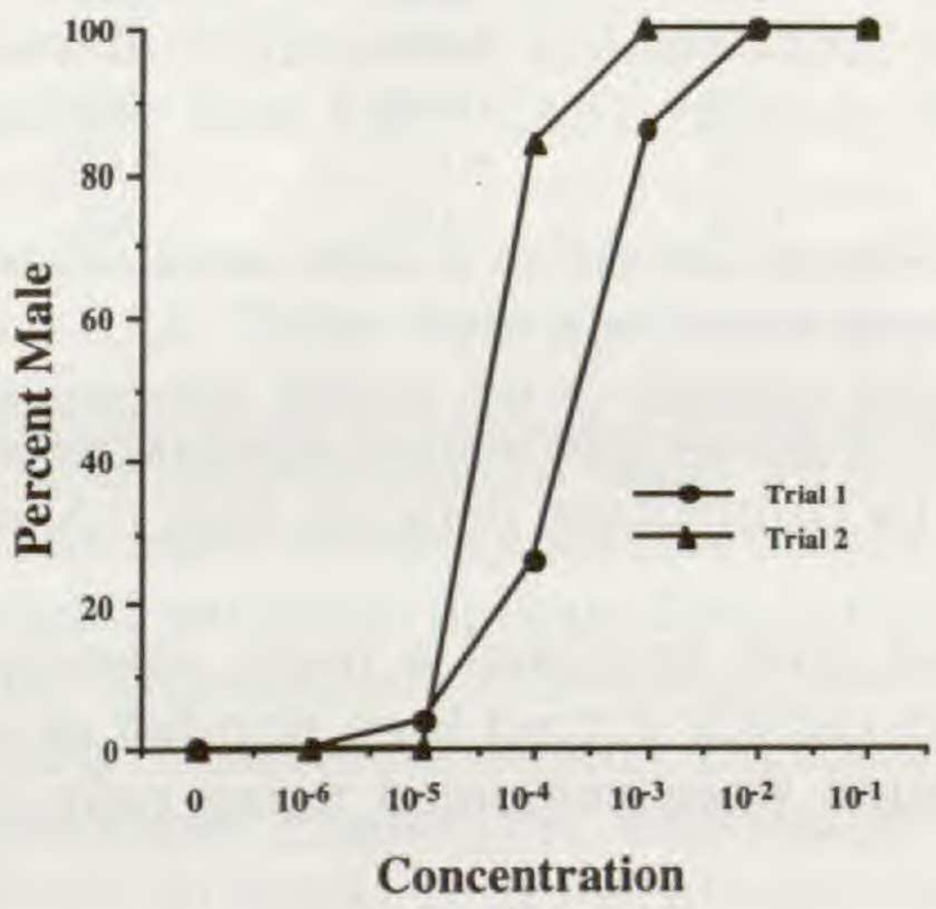
The relationship between gametophyte surface area and antheridium number was explored. To determine whether the number of antheridia per gametophyte increased with area, regression analysis was performed within each treatment (Table 3). Number of antheridia was positively associated with gametophyte surface area for all three replicates at 10^{-1} CAF, for all at 10^{-2}, two of the three at $10^{-3,}$ and two at 10^{-4}. In all other treatments, there was no significant relationship between area and antheridial number. Thus, significant regressions tended to be observed in cultures where most or all gametophytes possessed antheridia, indicating that for male gametophytes, number of antheridia increased with size of the gametophyte. However, the strength of this association was not great, the highest value of r^2 being 0.722. Nor was the

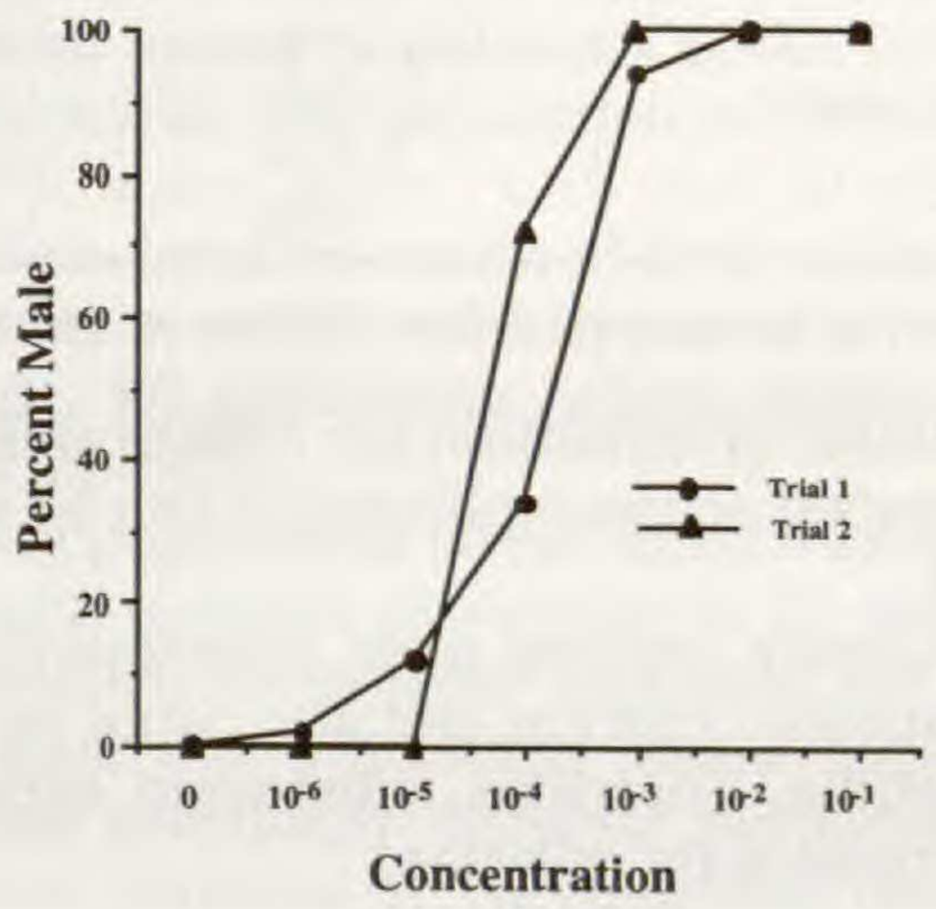
→

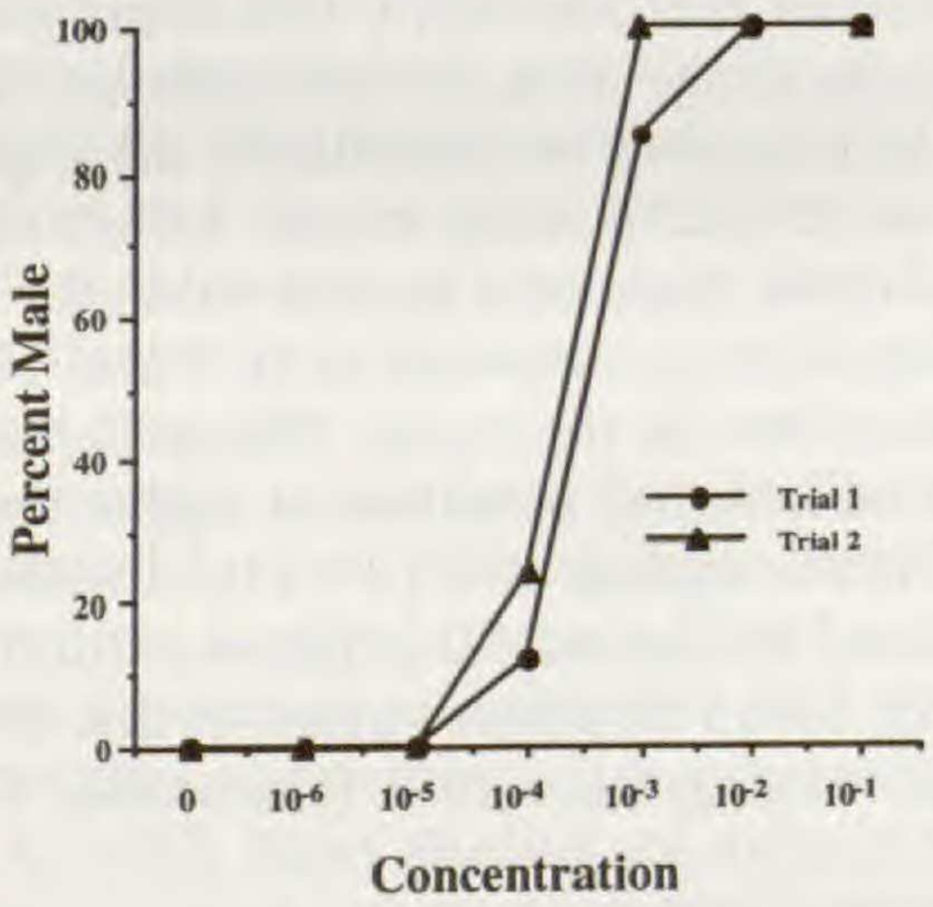
FIG. 2. Response of *Onoclea sensibilis* gametophytes from six populations to varied doses of antheridiogen A_{PT}, as characterized by percentage of male gametophytes.


Gray GA
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
Trial 3
0
10-5
10-4
10-3
10-2
10-1
Concentration


Eagle WI
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration


Richmond VT
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration


Berwick PA
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration


Nashville TN
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration

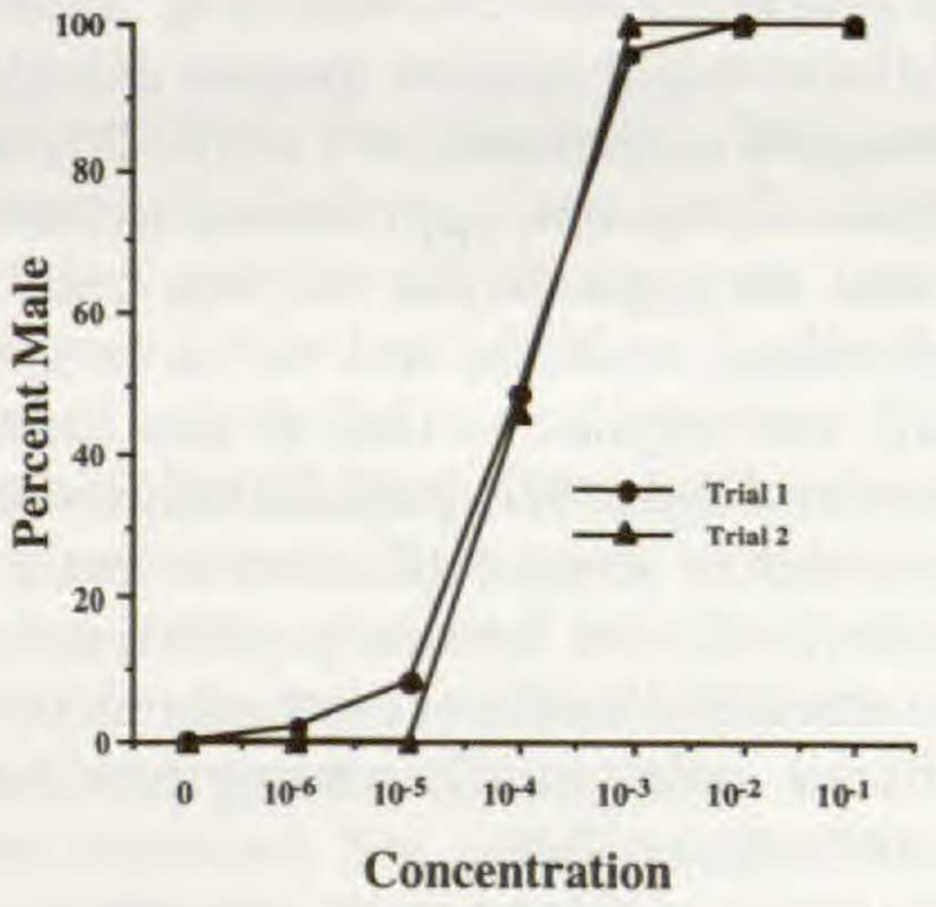

Vienna VA
Percent Male
100
80
60
40
20
0
Trial 1
Trial 2
0
10-6
10-5
10-4
10-3
10-2
10-1
Concentration

TABLE 3. Relationships between number of antheridia (X Anth.) and surface area (X Area) of gametophytes for each of the three replicates (Rep.) at each concentration of CAF (Treatment). The abbreviation r^2 refers to the regression coefficient, df to degrees of freedom, and P to resultant probability. Asterisks (*) next to r^2 values indicate a significant relationship at the $P < 0.05$ level.

Treatment	Rep.	X Anth.	X Area	df	r^2	P
10-1	1	40.50	0.72	1,28	0.704*	0.000
	2	37.77	0.65	1,28	0.559*	0.000
	3	19.90	0.60	1,28	0.722*	0.000
10-2	1	49.23	0.77	1,28	0.632*	0.000
	2	35.67	0.58	1,28	0.409*	0.000
	3	22.17	0.80	1,28	0.657*	0.000
10-3	1	34.67	1.14	1,28	0.005	0.725
	2	35.96	0.83	1,28	0.713*	0.000
	3	19.07	0.91	1,28	0.538*	0.000
10-4	1	16.77	1.68	1,28	0.138*	0.044
	2	15.40	1.73	1,28	0.158*	0.029
	3	9.87	1.05	1,28	0.009	0.619
10-5	1	0.03	1.92	1,28	0.051	0.231
	2	0.60	1.11	1,28	0.049	0.238
	3	0.00	1.23	1,28	0.000	NA
Control	1	0.00	1.23	1,28	0.000	NA
	2	0.10	1.53	1,28	0.101	0.086
	3	0.00	1.37	1,28	0.000	NA

number of antheridia per surface area unit of gametophyte even approximately constant across replicates, as can be seen by comparison between Figs. 1A and 1B.

Clearly, percent male gametophytes not only provided a more consistent response criterion, but also could be more rapidly scored than number of antheridia. Therefore, subsequent experiments were evaluated using only the percent male criterion.

COMPARISON OF POPULATIONS.—Five additional populations were compared to each other and to the Gray, GA, population for antheridiogen response using the percent male criterion (Fig. 2). To allow for the possibility that some populations might exhibit greater sensitivity, the range of A_{PT} concentration was extended to include 10^{-6} CAF. The level of response was similar in all populations. Response approached saturation at 10^{-3} CAF, with means across replicates for populations varying from 96.6–100% male (the lowest value for an individual replicate was 85%). In most populations, response at 10^{-5} and 10^{-6} CAF was similar to that of the control, i.e., few to no males. The exception was the Eagle, WI, population, in which substantial numbers of males were observed in some replicates at both 10^{-5} (21% males) and 10^{-6} (16% males); control cultures for this population contained no males. All cultures exhibited intermediate levels of response at 10^{-4} CAF. Mean response values at this dose differed substantially among populations, ranging from 18% (Nashville, TN) to 78% (Gray, GA).

Relative to the amount of variation among populations, there was a substan-

tial amount of variation in response at 10^{-4} between replicates for some populations. Variation in response among replicates was greatest in the Richmond, VT, population (26–84% males at 10^{-4} CAF) and least in the Gray, GA, population (68.9–79.3% males at 10^{-4} CAF).

Discussion

Herein we have quantified three components of response by *O. sensibilis* to varied concentration of antheridiogen A_{PT}. Percent males and mean number of antheridia per gametophyte increased and gametophyte surface area decreased with increasing doses of A_{PT}. Of these, percent male was the most predictable, and provides a consistent and convenient means to characterize antheridiogen response. Using this criterion, threshold and saturation doses of A_{PT} concentration were found to differ by approximately two orders of magnitude (ca. 10^{-5} and 10^{-3} CAF, respectively), with the intermediate concentration (10^{-4} CAF) eliciting an intermediate response. This response profile is by and large consistent among populations sampled across the species range. However, there is evidence of at least some variation among populations at the intermediate dose (10^{-4} CAF). Notably, the Nashville, TN, population exhibited a substantially lower response (mean of 18% males) at 10^{-4} CAF than all other populations, which exhibited mean response levels in excess of 50% males at 10^{-4} CAF. There were also some populations in which males appeared at concentrations below 10^{-4}, notably the Eagle, WI population, which exhibited substantial numbers of males at 10^{-5} and 10^{-6} CAF.

The fundamentally similar results among populations could reflect a lack of genetic variation determining antheridiogen-response phenotype in *O. sensibilis*. Alternatively, substantial genetic variation for response may exist, but be evenly distributed among populations such that population means appear equivalent. Phenotypic differences among genetically different individuals would be small if antheridiogen response variation is polygenically determined. It is uncertain whether the differences in response by individual gametophytes exhibited at 10^{-4} CAF are a result of genetic differences among gametophytes or instead represent stochastic response variations among gametophytes that are genetically uniform with respect to determinants of A_{PT} response. The lower response value at 10^{-4} CAF in the Nashville, TN, population and the occasional response of some gametophytes (e.g., in the Eagle, WI, population) at extremely low doses (10^{-5} and 10^{-6} CAF) suggests that at least some genetic variation may exist. However, the heritability of antheridiogen response is completely unknown in *O. sensibilis* or in any other polypodiaceous (sensu lato) fern. In *Ceratopteris richardii* (Parkeriaceae), mutation-induced lack of antheridiogen sensitivity was shown to have a simple (two gene) mode of inheritance (Scott and Hickok, 1987; Warne et al., 1988). However, it remains possible that this species includes more subtly differentiated, continuously varying phenotypes that are determined polygenically.

As with most studies on antheridiogen response, the present experiments were carried out under highly artificial conditions that constrain their rele-

vance to the natural life history characteristics of *O. sensibilis*. Unnatural conditions included use of agar solidifed media instead of soil, heat sterilization of media and antheridiogen via autoclaving at temperatures greatly exceeding those encountered in nature, continuous illumination, and use of antheridiogen derived from bracken rather than from *Onoclea* itself. Use of definable artificial conditions make such experiments more feasible, repeatable, and comparable to the broad literature which has used similar conditions. The validity of this and other similar experiments and relevance to nature could be evaluated by repeating them under more natural conditions, as has been done but rarely (Haufler and Ranker, 1985).

Irrespective of whether the present results reflect life-history attributes of *O. sensibilis,* they do have bearing on the use of this species as a standard assay organism for detecting antheridogen A. The similar response profile observed across a large portion of the species range further validates the reliability and consistency of *O. sensibilis* for this purpose. The means by which antheridiogen response is quantified has varied among studies, having included determining the minimum dose that can produce any response (e.g., Näf, 1956; Näf et al., 1975), percentage of males in cultures (Näf, 1965; Klekowski and Lloyd, 1968; Schedlbauer and Klekowski, 1972; Rubin and Paolillo, 1983; Scott and Hickok, 1987; Nester-Hudson et al., 1997), or number of antheridia per gametophyte (Haufler and Ranker, 1985). The response variation among populations at the lower concentrations indicates that inconsistencies may be experienced using the minimal response criterion, unless the same spore source of *Onoclea* is always used. We found that the most easily scored response attribute, percent male, is also the most reliable for judging A_{PT} concentration. The strength of A_{PT} extracts could be standardized by determining the dilution at which 50% of the gametophytes are male, analogous to the LD50 criterion used in toxicology. Moreover, the discovery of individuals such as the one from Nashville, TN, with lower sensitivity could provide an additional assay point for more precisely standardizing antheridogen concentrations. Such standardization will have importance as research on antheridiogen response continues, as different CAF extractions may vary in their A_{PT} concentration and diminish over time.

Acknowledgments

We are grateful to Paul Wolf for providing bracken spores, to Ralph Brooks, Carol Kuhn, Chris Haufler, John Miller, Carl Taylor, James Montgomery, Dean Whittier, David Barrington, and Cathy Paris for providing collections of *Onoclea* sporophylls, and to Lisa Wellborn for help in developing methodology. Helpful comments by an anonymous reviewer resulted in improvement of the manuscript. This research was supported by a REU supplement to NSF grant BSR-851184 and by DEB-9220755.

Literature Cited

Döpp, W. 1950. Ein die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* (L.) Kuhn. Ber. Deutsch. Bot. Ges. 63:139–147.

GASTONY, G. J., and M. C. UNGERER. 1997. Molecular systematics and a revised taxonomy of the Onocleoid ferns (Dryopteridaceae: Onocleae). Amer. J. Bot. 84:840–849.

HAUFLER, C. H., and G. J. GASTONY. 1978. Antheridiogen and the breeding system in the fern genus *Bommeria*. Canad. J. Bot. 56:1594–1601.

HAUFLER, C. H., and T. A. RANKER. 1985. Differential antheridiogen response and evolutionary mechanisms in *Cystopteris*. Amer. J. Bot. 72:659–665.

KLEKOWSKI, E. J. JR. 1969. Reproductive biology of the pteridophyta. III. A study of the Blechnaceae. Bot. J. Linn. Soc. 62:361–377.

KLEKOWSKI, E. J. JR., and R. M. LLOYD. 1968. Reproductive biology of the pteridophyta. 1. General considerations and a study of *Onoclea sensibilis* L. J. Linn. Soc., Bot. 60:315–324.

MILLER, J. H. 1968. Fern gametophytes as experimental material. Bot. Rev. (Lancaster) 34:361–426.

MILLER, J. H., and P. M. MILLER. 1970. Unusual dark-growth and antheridial differentiaion in some gametophytes of the fern *Onoclea sensibilis*. Amer. J. Bot. 57:1245–1248.

NÄF, U. 1956. The demonstration of a factor concerned with the initiation of antheridia in Polypodiaceous ferns. Growth 20:91–105.

———. 1965. On antheridial metabolism in the fern species *Onoclea sensibilis* L. Pl. Physiol. 40: 888–890.

———. 1979. Antheridiogens and antheridial development. Pp. 435–468 *in* A. F. Dyer, ed. *The experimental biology of ferns*. Academic Press, London.

NÄF, U., K. NAKANISHI, and M. ENDO. 1975. On the physiology and chemistry of fern antheridiogens. Bot. Rev. (Lancaster) 41:315–359.

NESTER-HUDSON, J. E., C. LADAS, and A. MCCLURD. 1997. Gametophyte development and antheridiogen activity in *Thelypteris ovata* var. *lindheimeri*. Amer. Fern J. 87:131–142.

PECK, J. H., C. J. PECK, and D. R. FARRAR. 1990. Influences of life history events on formation of local and distant fern populations. Amer. Fern J. 80:126–142.

RANKER, T. A. 1987. Experimental systematics and population biology of the fern genera *Hemionitis* and *Gymnopteris* with reference to *Bommeria*. Ph. D. dissertation, University of Kansas, Lawrence.

RUBIN, G., and D. J. PAOLILLO. 1983. Sexual development in *Onoclea sensibilis* on agar and soil media without the addition of antheridiogen. Amer. J. Bot. 70:811–815.

SCHEDLBAUER, M. D., and E. J. KLEKOWSKI JR. 1972 Antheridiogen activity in the fern *Ceratopteris thalictroides* (L.) Brogn. Bot. J. Linn. Soc. 65:399–413.

SCHNELLER, J. J., C. H. HAUFLER, and T. A. RANKER. 1990. Antheridiogens and natural gametophyte populations. Amer. Fern J. 80:143–152.

SCOTT, R. J., and L. G. HICKOCK. 1987. Genetic analysis of antheridiogen sensitivity in *Ceratopteris richardii*. Amer. J. Bot. 74:1872–1877.

VOELLER, B. R. 1964. Antheridiogens in ferns. Colloq. Intern. Centre Nat. Rech. Sci. (Paris) 123: 665–684.

WARNE, T. R., L. G. HICKOK, and R. J. SCOTT. 1988. Characterization and genetic analysis of antheridiogen-insensitive mutants in the fern *Ceratopteris*. Bot. J. Linn. Soc. 96:371–379.

YATSKIEVYCH, G. 1993. Antheridiogen response in *Phanerophlebia* and related ferns. Amer. Fern J. 83: 30–36.